ABSTRACT

Harvesting the full richness of biodiversity is instantly recognized by Diversa Corporation as a powerful means to access both novel molecules having direct commercial utility as well as molecular templates that could be retooled to acquire commercial utility. A directed evolution process for rapid and facilitated production from a progenitor polynucleotide template, of a library of mutagenized progeny polynucleotides wherein each of the 20 naturally encoded amino acids is encoded at each original codon position. This method, termed site-saturation mutagenesis, or simply saturation mutagenesis, is preferably based on the use of the degenerate N,N,G/T sequence. Also, a method of non-stochastically producing a library of chimeric nucleic acid molecules having an overall assembly order that is chosen by design. Accordingly, a set of progenitor templates, such as genes (e.g. a family of esterase genes) or genes pathways (e.g. encoding antibiotics) can be shuffled to generate a sizable library of distinct progeny polynucleotide molecules (e.g. 10^{100}) and correspondingly encoded polypeptides. Screening of these polynucleotide libraries enables the identification of a desirable molecular species that has a desirable property, such as a specific enzymatic activity serviceable for a commercial application, or a novel antibiotic. Also, a method of retooling genes and gene pathways by the introduction of regulatory sequences, such as promoters, that are operable in an intended host, thus conferring operability to a novel gene pathway when it is introduced into an intended host. For example a novel man-made gene pathway , generated based on microbially-derived progenitor templates, that is operable in a plant cell.